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Design and Evaluation of Microspheres Loaded With Pirenzepine

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ABSTRACT

The current objective of the investigation was to fabricate Pirenzepine loaded microspheres for the treatment of gastritis delivered through oral route. The microspheres were prepared by ionotropic gelation technique using sodium as alginate polymer and calcium chloride as cross-linking agent. The effect of polymer and cross-linking agent on particle size, shape, % yield, entrapment efficiency, and drug release were studied. The prepared microspheres morphology and physicochemical properties of were investigated by Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Among the total S14 formulations, S7 formulation was optimized at 2.2% of sodium alginate, 7% of calcium chloride maintained100rpm for 10 min at room temperature. The optimized S7 formulation displayed the %EE 94.10%, particle size 82.45 ± 0.09µm, % yield 96.30% and swelling index of 95.13%. From *In vitro* drug release studies S7 shown 97.17 ± 0.28% up to 12 h in 0.1N HCl, and the drug release followed the zero order and Korsmeyer-Peppas model (R^2 = 0.987, 0.995) respectively, indicating the possible drug release mechanism to be by erosion and diffusion. The marketed product showed the drug release of 95.23 ± 0.21% within 1 h. The optimized S7 formulation subjected to stability studies for 6months as per ICH guidelines, no appreciable difference was observed hence the S7 formulation found stable. The data obtained thus suggest that a micro particulate system can be successfully designed for sustained delivery of Pirenzepine and to improve its bioavailability.

Keywords: Microspheres, Pirenzepine, Release kinetics, Gastritis, SEM.

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INTRODUCTION

Gastritis is a chronic inflammatory disease of the stomach mucosa. Nowadays, the prevalence and incidence of gastritis is increasing, especially in developed countries. [1] There is need to develop new drugs and novel formulations as alternative to existing

formulations. But bringing an innovative drug molecule from discovery to commercialization is a tough, lengthy and costly process. Hence, the formulation scientist has been carrying out the research on improving efficacy and reducing toxicity of old drugs by the development of new drug delivery techniques such as micro particulates. [2] The major conventional therapeutic strategies are to reduce inflammatory episodes, but dosing frequency is high. Hence, the rapid and extensive drug absorption is required in the upper gastrointestinal tract (GIT), which resulted in better therapeutic effect and the lower side effects had improved to its application. [3]

Microspheres are defined as monolithic solid spherical particles in the size range of about 1-1000µm. Microspheres are potential drug carriers for oral controlled release. Microspheres had significant importance in biomedical applications. [4] Microspheres can be produced by using sodium alginate as polymer and calcium chloride as cross-linking agent. Drug administration in the form of microspheres usually enhances the treatment by providing the drug substance at the site of action and by sustaining drug release finally reduces gastric irritation. [5] There are several encapsulation methods; among those ionic gelation is an interesting method, given its simplicity and versatility. [6]

Pirenzepine is a newer antimuscarinic agent (M1) used to inhibit the gastric secretion. The drug candidate associated with low bioavailability (25%) hence is rapidly metabolized into its inactive metabolite within liver and colonic environment so the efficacy would be reduced and requires multiple dosing for maintaining therapeutic effect throughout the day. One approach to avoid this problem would be control the drug release hence increases the bioavailability at insitu level. [7]

Polymeric drug delivery system displays several advantages over the conventional dosage forms and it includes enhanced efficacy, patient compliance, reduced toxicity, and to control the encapsulated drug release. [8] Sodium alginate is anionic natural polysaccharide, prepared by mixture of D-mannuronic acid and L-glucuronic acid. Sodium alginate is extensively used as carrier for drug delivery due to its biocompatibility and low toxicity. [9] The widely used method for microspheres preparation is an ionotropic gelation method. This technique offers several advantages such as simple method of preparation no need to use of organic solvent, and, also easier to control. Sodium alginate could form gel in the presence of multivalent cations such as Ca^{2+}, Zn^{2+}, Ba^{2+} and Al^{3+} etc... by ionic cross-linking to form microspheres, it has been widely used in sustained drug release. Hence in this study calcium chloride is selected as cross-linking agent and because of its nontoxic and biocompatibility. [10]

The aim of the present study is to develop Pirenzepine loaded microspheres by ionotropic gelation method to obtain an extended retention in the upper GIT, which may result in increased absorption and thereby improved bioavailability. The prepared microspheres were evaluated for particle size, shape, % yield, incorporation efficiency, and *in vitro* release study.

Table 1: Formulation trials for Pirenzepine normal microspheres

Formulation code	Pirenzepine (mg)	Sodium alginate	Calcium chloride
S1	50	1%	7%
S2	50	1.2 %	7%
S3	50	1.4%	7%
S4	50	1.6%	7%
S5	50	1.8%	7%
S6	50	2%	7%
S7	50	2.2%	7%
S8	50	1%	10%
S9	50	1.2%	10%
S10	50	1.4%	10%
S11	50	1.6%	10%
S12	50	1.8%	10%
S13	50	2%	10%
S14	50	2.2%	10%

MATERIALS AND METHODS

MATERIALS

Pirenzepine was obtained from Splendid laboratories, Pune, India as a gift sample. Sodium alginate was purchased from Pruthvi Chemicals, Mumbai, India, calcium chloride was obtained from SD Fine ltd, Mumbai., India. Remaining all chemicals used in this research study was of analytical grade.

METHODS

Preparation of Pirenzepine microspheres

Microspheres were prepared by the ionotropic gelation technique using various percentages of sodium alginate was ranges from 1% to 2.2% w/v and calcium chloride at 7% and 10% as mentioned in Table 1. Initially, sodium alginate solution (100 ml) was prepared in to that dissolved the weighed quantity of Pirenzepine (50 mg) at room temperature. The above dispersion was sonicated for 30 min to eliminate air bubbles that may have been formed during the stirring process. The above dispersions (100 ml) was added drop wise via a 20-gauge needle fitted with a 10 ml syringe into 100 ml of 7% w/v and 10% w/v of calcium chloride solution, being stirred at 500 rpm for 10 min. The formed microspheres were collected by filtration, washed repeatedly with distilled water, and dried at 60°C for 2 h in a hot air oven. [11]

Evaluation of Pirenzepine microspheres

Size analysis

Microsphere Size plays significant role in determining the drug release from it. Particle size analysis was made by optical microscopy technique, using calibrated eye piece and a stage micrometer, almost 100 particles were measured. [12]

Flow properties

Flow properties of the prepared microspheres were determined in terms of Angle of repose, Bulk density, tapped density, Compressibility index and Hausner's ratio according to the reported method. [13]

Swelling index studies

The capacity of the microspheres to absorb water and swell was determined in terms of swelling index. For determining swelling index, the microspheres were weighed initially then suspended in pH 1.2. After 1 h microspheres were transferred onto blotting paper to remove the excess moisture then weighed the swollen microspheres using a microbalance. After that swollen microspheres were dried in oven at 60°C for 5 h until showed the constant weight. The difference in weight of microspheres was used to calculate the swelling index. [14]

Swelling index= (Mass of swollen microspheres - Mass of dry microspheres/mass of dried microspheres) 100.

Drug incorporation efficiency and % yield

To determine the %EE, microspheres (10 mg) were weighed, carefully crushed, triturated and suspended in a required quantity of methanol for dissolving microspheres shell coat. The suspension was suitably diluted with water and filtered to separate shell fragments. The drug content was analyzed after suitable dilution spectrophotometrically at 280 nm. [15] The amount of drug incorporated in microspheres was calculated by the following formula

% Drug entrapment = Calculated drug concentration /Theoretical drug concentration × 100

And % yield is calculated by the following formula

% yield = [Total weight of microspheres / Total weight of drug and polymer] × 100

In vitro drug release studies

In the current study, drug release from microspheres was studied using USP Type 2 (paddle) dissolution apparatus at 100 rpm in 0.1N HCl (pH 1.2) as dissolution fluids (900 ml) maintained at 37 ± 0.5°C. The samples were withdrawn at predetermined time intervals such as 1, 2, 4, 6, 8, 10 and 12 h simultaneously same volume replenished each time to maintain the sink condition. The samples were analyzed spectrophotometrically at 280 nm for the estimation of Pirenzepine concentrations in the test samples. [16] All experiments were conducted in triplicate.

Kinetic modeling of drug release

The optimized formulation (S7) was treated with the different release kinetic equations include Zero order, First order, Higuchi's model and Korsmeyer-Peppas. Analysis of drug release from microspheres was determined by calculating the (r^2) correlation coefficient.

Drug excipient compatibility studies

Drug-excipient compatibility was studied by Fourier transmission infrared spectroscopy (FTIR) and Scanning electron microscopy (SEM).

Fourier transform infrared spectroscopy (FTIR)

The FTIR technique can be used to recognize the functional groups in the sample and drug-excipient compatibility. FTIR spectra of pure Pirenzepine, physical mixtures and optimized formulation were recorded by using FTIR (SHIMADZU). Weighed quantity of KBr and excipients were taken in the ratio 100: 1 and mixed by mortar. The samples were made into pellet/disk by the application of pressure. [17] Then the FTIR spectra were recorded between 400 to 4000 cm^{-1}.

Differential Scanning Calorimetry (DSC)

The Differential Scanning Calorimetry measurements were performed on DSC-60 associated with TA-60 software. Accurately weighed Samples were placed in aluminum pan and sealed before heating under nitrogen flow at a scanning rate of 10°C min^{-1} from 25°C to 350°C. An empty aluminum pan was used as reference. [18]

SEM studies

Surface nature of microspheres includes size and shape was examined with the help of Scanning Electron Microscope (HITACHI, S-3700N). The microspheres were dried completely prior to analysis and SEM was carried out at different magnifications of 15.0 kv × 7.1 mm, 15 kv × 6.7 mm, 15Kv × 6.9 mm. [19]

Stability studies

Stability studies were conducted at 40°C ± 2°C/75% RH ± 5% RH for 6 months using stability chamber (Thermo Lab, Mumbai). Samples were withdrawn at predetermined intervals of 0, 30, 60, 120, and 180 days period according to ICH guidelines. Various *in vitro* parameters like % yield, entrapment efficiency and *in vitro* release studies were evaluated. [20]

Fig. 1: **Pirenzepine microspheres**

RESULTS AND DISCUSSIONS

Micromeretic properties of Pirenzepine microspheres

The results of Micromeretic parameters and swelling index of Pirenzepine microspheres were summarized in Table 2. The prepared microspheres were shown in pictorial diagram, Fig. 1. All the formulations particle size was in the range of 61.12 ± 0.08 to 94.13 ± 0.09µm. Bulk and tapped densities of S1-S14 was in the range of 0.60 - 0.89g/cc^3, 0.59 - 0.78 g/cc^3 respectively, indicated good packability of the microspheres. The angle of repose value of S1-S14 was in the range of 20˚.54 - 30˚.54 which showed excellent to good flowing nature of the formulated microspheres. Carr's index ranges from 8.12% to 14.56%.

Table 2: Micromeritic properties of Pirenzepine microspheres

Formulation code	Particle size (µm)	Bulk density (g/cc³)	Tapped density (g/cc³)	Angle of repose	Carr's index	Swelling index (%)
S1	61.12 ± 0.08	0.66 ± 0.02	0.69 ± 0.15	23˚.74 ± 0.02	9.34 ± 0.12	64.23 ± 0.12
S2	87.29 ± 0.13	0.74 ± 0.15	0.72 ± 0.27	29˚.67 ± 0.12	10.34 ± 0.27	69.6 ± 0.33
S3	73.45 ± 0.04	0.80 ± 0.28	0.60 ± 0.33	30˚.54 ± 0.17	9.78 ± 0.31	70.12 ± 0.16
S4	69.67 ± 0.09	0.79 ± 0.07	0.73 ± 0.16	26˚.15 ± 0.39	11.36 ± 0.16	71.56 ± 0.20
S5	92.45 ± 0.09	0.62 ± 0.31	0.75 ± 0.22	27˚.93 ± 0.26	14.52 ± 0.20	79.76 ± 0.37
S6	67.43 ± 0.04	0.76 ± 0.20	0.64 ± 0.31	25˚.21 ± 0.15	13.95 ± 0.11	87.98 ± 0.15
S7	82.45 ± 0.09	0.60 ± 0.17	0.59 ± 0.14	20˚.54 ± 0.26	8.12 ± 0.09	95.13 ± 0.22
S8	67.45 ± 0.04	0.71 ± 0.32	0.73 ± 0.38	27˚.93 ± 0.33	14.56 ± 0.12	69.78 ± 0.32
S9	78.45 ± 0.09	0.67 ± 0.11	0.62 ± 0.22	25˚.54 ± 0.19	13.95 ± 0.24	70.91 ± 0.11
S10	81.23 ± 0.14	0.74 ± 0.23	0.76 ± 0.15	24˚.91 ± 0.22	10.32 ± 0.38	75.60 ± 0.27
S11	94.13 ± 0.09	0.66 ± 0.34	0.66 ± 0.36	23˚.74 ± 0.34	9.34 ± 0.27	84.97 ± 0.30
S12	65.29 ± 0.13	0.87 ± 0.16	0.73 ± 0.27	25˚.67 ± 0.25	12.34 ± 0.17	93.72 ± 0.18
S13	91.43 ± 0.04	0.76 ± 0.25	0.68 ± 0.12	29˚.15 ± 0.18	10.12 ± 0.13	92.54 ± 0.22
S14	85.12 ± 0.08	0.69 ± 0.39	0.78 ± 0.08	25˚.54 ± 0.27	11.65 ± 0.4	89.51 ± 0.30

The swelling index was in the range of 64.23% to 95.13%. Comparatively, S7 formulation showed excellent micromeritic results this suggests that the optimized microspheres (S7) easily handled during processing.

Entrapment efficiency and % yield

The % EE and Percentage yield of all formulations varies from 69.27% to 94.10% and 70.15% to 96.30%, respectively, the best one was S7 as given in Table 3. It was resulted that % yield increased with an increase the polymer percentage. In some formulations observed the low % yield may be due to leakage of drug from the microspheres during washing process. It was also showed that as polymer % in the formulation increased, the percentage entrapment efficiency also increased this might be due to an increase in the entrapment of drug in the swollen structure of sodium alginate.

In vitro drug release studies

In vitro release studies were conducted, and the results are shown in Table 4 and 5 and in Fig. 2 and 3. The Cumulative release of Pirenzepine significantly decreased with increasing polymer concentration. The optimized formulation S7 showed the drug release 97.17 ± 0.28% within 12 h whereas drug release from marketed product was 95.23 ± 0.21% within 1 h.

The *in vitro* release data was fitted into equations for the Zero order, first order, Higuchi model and Korsmeyer-Peppas model and results were mentioned in the Table 6. The results were showed the highest regression coefficient (R^2) values for Zero order (R^2=0.987) and Korsmeyer-Peppas model (R^2=0.995). The n-value was 0.836 indicating erosion and diffusion to be the principal mechanism of drug release.

Table 3: Percentage drug yield, entrapment efficiency of Pirenzepine microspheres

Formulation code	Percentage yield (%)	Entrapment efficiency (%)
S1	70.15 ± 0.07	69.27 ± 0.08
S2	83.87 ± 0.15	83.30 ± 0.21
S3	81.28 ± 0.24	91.30 ± 0.33
S4	91.30 ± 0.31	72.18 ± 0.17
S5	86.28 ± 0.19	85.20 ± 0.26
S6	71.24 ± 0.22	80.39 ± 0.30
S7	96.30 ± 0.37	94.10 ± 0.22
S8	84.75 ± 0.07	83.52 ± 0.18
S9	81.92 ± 0.21	82.72 ± 0.31
S10	76.38 ± 0.35	91.03 ± 0.29
S11	86.09 ± 0.28	85.19 ± 0.16
S12	93.92 ± 0.11	86.66 ± 0.27
S13	87.50 ± 0.29	74.03 ± 0.37
S14	85.76 ± 0.32	84.88 ± 0.22

Table 4: *In vitro* Cumulative % drug release of Pirenzepine microsphere formulations from S1-S7 and Marketed product

Time (h)	S1	S2	S3	S4	S5	S6	S7	Marketed product
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	15.09 ± 0.15	14.87 ± 0.17	14.23 ± 0.15	13.23 ± 0.22	12.34 ± 0.22	11.31 ± 0.21	10.10 ± 0.16	95.23 ± 0.21
2	23.08 ± 0.21	22.06 ± 0.15	21.12 ± 0.15	20.12 ± 0.21	20.34 ± 0.24	21.15 ± 0.24	20.30 ± 0.21	--
4	39.11 ± 0.22	38.20 ± 0.11	38.90 ± 0.15	44.40 ± 0.21	46.20 ± 0.24	47.23 ± 0.21	49.40 ± 0.24	---
6	45.39 ± 0.15	48.30 ± 0.14	49.90 ± 0.15	51.70 ± 0.21	51.30 ± 0.21	56.73 ± 0.25	63.80 ± 0.21	---
8	56.23 ± 0.21	58.30 ± 0.21	61.21 ± 0.12	60.30 ± 0.22	63.30 ± 0.25	66.46 ± 0.15	72.60 ± 0.22	---
10	66.20 ± 0.14	69.90 ± 0.22	71.22 ± 0.16	70.30 ± 0.21	73.30 ± 0.21	81.45 ± 0.12	83.90 ± 0.27	---
12	70.34 ± 0.12	72.30 ± 0.21	80.20 ± 0.14	83.50 ± 0.21	86.30 ± 0.21	92.12 ± 0.23	97.17 ± 0.28	---

Table 5: *In vitro* Cumulative % drug Pirenzepine microsphere formulations from S8-S14

Time (h)	S8	S9	S10	S11	S12	S13	S14
0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
1	10.05 ± 0.12	13.23 ± 0.15	14.23 ± 0.15	15.00 ± 0.15	15.22 ± 0.15	15.62 ± 0.32	14.63 ± 0.21
2	21.40 ± 0.21	23.34 ± 0.16	24.80 ± 0.21	25.40 ± 0.19	24.23 ± 0.12	23.01 ± 0.16	32.01 ± 0.22
4	38.20 ± 0.22	38.90 ± 0.14	44.40 ± 0.23	38.20 ± 0.15	40.10 ± 0.23	38.24 ± 0.15	44.83 ± 0.21
6	48.30 ± 0.23	49.91 ± 0.23	51.60 ± 0.21	51.30 ± 0.18	54.20 ± 0.24	52.83 ± 0.21	57.76 ± 0.15
8	57.35 ± 0.24	61.20 ± 0.21	60.30 ± 0.22	63.30 ± 0.15	68.24 ± 0.21	67.03 ± 0.24	64.60 ± 0.18
10	69.90 ± 0.21	70.10 ± 0.14	70.60 ± 0.25	69.92 ± 0.16	72.32 ± 0.15	82.22 ± 0.22	75.56 ± 0.19
12	72.30 ± 0.22	80.20 ± 0.12	83.50 ± 0.21	85.42 ± 0.16	86.41 ± 0.16	94.36 ± 0.21	85.00 ± 0.21

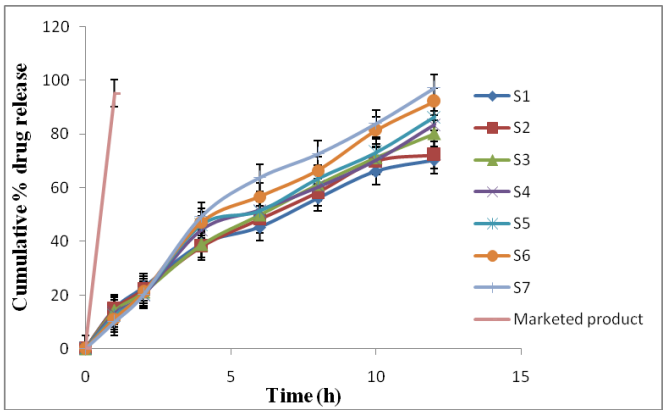

Fig. 2: *In vitro* **Cumulative % drug release of Pirenzepine microsphere formulations from S1-S7 and marketed product**

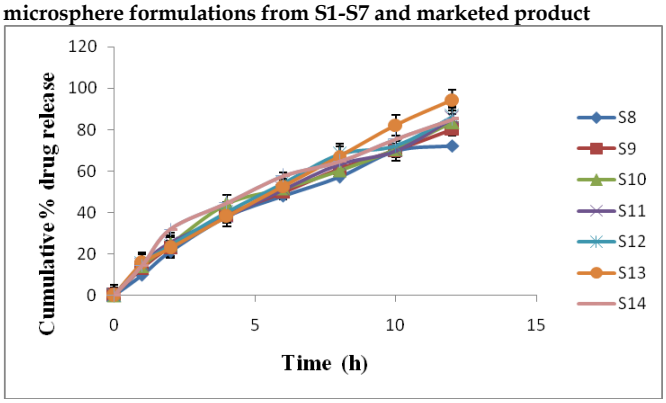

Fig. 3: *In vitro* **Cumulative % drug Pirenzepine microsphere formulations S8-S14 Mathematical modeling of optimized formulation (S7)**

Table 6: Release order kinetics of optimized normal microspheres (S7)

Formula Code	Zero Order	First Order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
S7	0.987	0.858	0.979	0.995	0.836

Drug excipient compatibility studies

FTIR spectroscopy of Pirenzepine microspheres

FTIR spectrumof pure drug characteristic sharp peaks of alkene stretching (=C-H and CH_2) vibration at 3324.32-3016.48 cm^{-1} and alkane stretching (-CH_3, -CH_2 and -CH) vibration at 2853.73 cm^{-1}. Also exhibited C=O stretch at 1738.2 cm^{-1} due to saturated ketone and C=O-NH stretching at 1635.90 cm^{-1}.

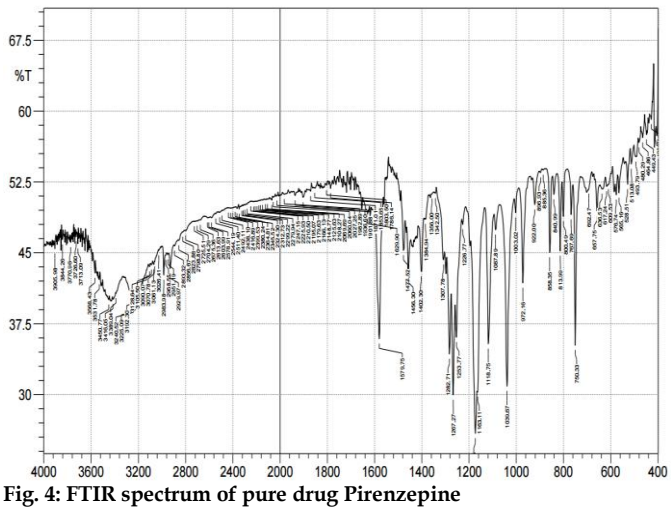

Fig. 4: FTIR spectrum of pure drug Pirenzepine

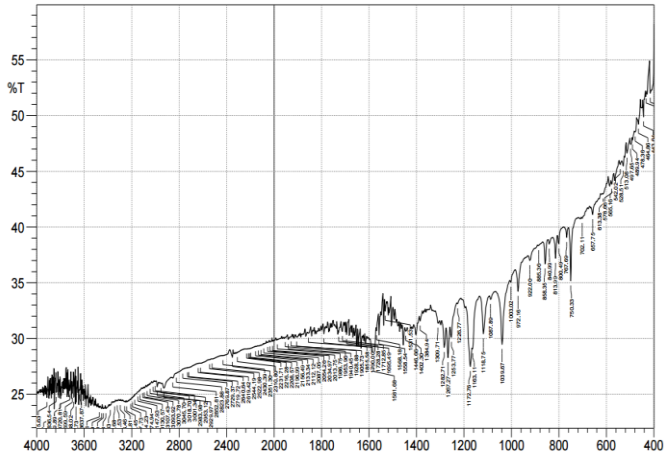

Fig. 5: FTIR spectrum of Pirenzepine optimized formulation S7

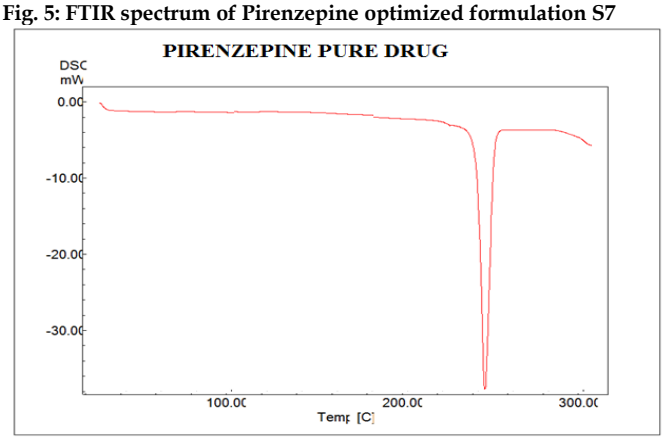

Fig. 6: DSC thermogram of Pirenzepine pure drug

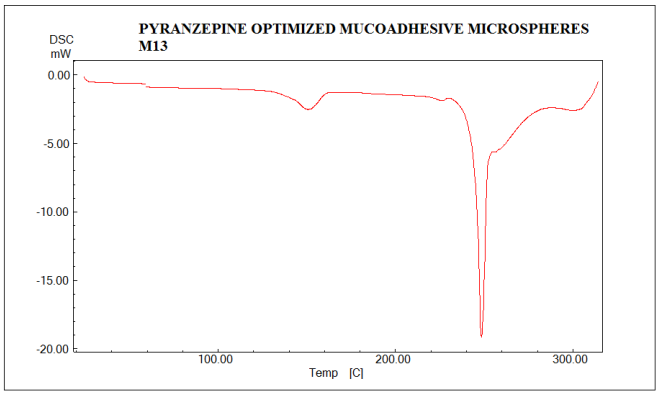

Fig. 7: DSC thermogram of Pirenzepine optimized microspheres S7

A selective stretching vibration at 1561.57 cm^{-1} and 1525.80 cm^{-1} for primary and secondary amine was also observed. For functional groups like S=O stretch and -C-S stretch showed vibrations at 1041.78 cm^{-1} and 729.57 cm^{-1} respectively (Fig. 4). There were no new significant bands observed in the pure drug (Fig. 4) and optimized formulation (Fig. 5), which confirms that no interaction takes place between the drug excipients.

DSC studies

DSC thermograms of pure drug, and optimized formulation (S7) were recorded with reference as a function of temperature shown in Figure 6 & 7. The peak corresponding to the melting of Pirenzepine (250°C) was appeared in the thermogram of optimized formulation (252°C). Presence of melting endotherm in the optimized formulation (Figure 7) indicated no significant interaction between drug and excipients.

SEM studies of Pirenzepine microspheres

SEM was performed for the optimized Pirenzepine microspheres (S7) to access their surface and morphological characteristics as shown in Fig. 8. The results found that microspheres were almost spherical and free-flowing.

Stability studies

From the stability studies it was observed that there was no significant change in results before and after stability studies hence the optimized formulation S7 found to be stable (Table 7).

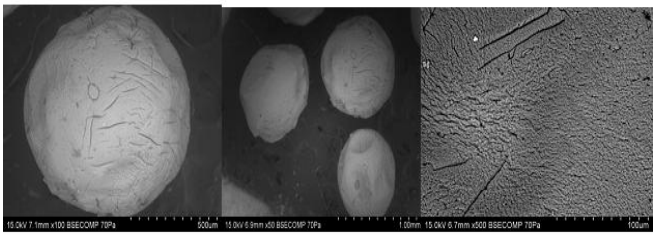

Fig. 8: Scanning electron micrographs of Pirenzepine microspheres

Table 7: Stability studies of optimized Pirenzepine microspheres

Retest Time for Optimized formulation	% yield	% Entrapment efficiency	*In-vitro* drug release profile (%)
0 days	96.30 ± 0.03	94.10 ± 0.02	97.17 ± 0.05
30 days	94.40 ± 0.04	93.46 ± 0.05	95.20 ± 0.07
60 days	95.22 ± 0.07	92.53 ± 0.06	94.33 ± 0.09
120 days	95.13 ± 0.09	91.55 ± 0.08	96.68 ± 0.07
180 days	93.34 ± 0.07	93.45 ± 0.05	95.56 ± 0.02

In this study, stable sustained release Pirenzepine microspheres were prepared successfully by the ionotropic gelation method using sodium alginate (polymer) and calcium chloride (cross linking agent). The Cumulative % drug release was found to be slow, controlled release over a period of 12 h when compared to marketed product. The drug release followed the zero order and Higuchi model indicated the release was controlled by diffusion. Accelerated stability studies confirmed that the microspheres formed were stable. The results of the present study indicated promising potential of microspheres in the delivery of drugs have lower bioavailability with controlled release of Pirenzepine in the management of gastritis.

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